

13 Biochemistry of Herbicide Decomposition in Soils

P. C. KEARNEY and D. D. KAUFMAN

*Crops Research Division
Agricultural Research Service
U.S. Department of Agriculture
Beltsville, Maryland*

M. ALEXANDER

*Department of Agronomy
Cornell University
Ithaca, New York*

13-1. Introduction.....	318
13-2. Phenoxyalkanoic Acids	319
13-3. Phenylcarbamates	323
13-4. Phenylureas	326
13-5. Chlorinated Aliphatic Acids	329
13-6. s-Triazines	331
13-7. Toluidines and Acetamides	334
13-8. Amitrole.....	336
13-9. Dipyridyls.....	336
13-10. Ecology and Microbiology of Herbicide Degradation	337
13-11. Conclusion.....	339
References.....	340

13-1. INTRODUCTION

The fate and behavior of pesticides in nature are receiving major research emphasis in many universities and government agencies. Agricultural soils are the ultimate recipient and depository of most applied foliar and soil pesticides. Because of the public health significance of pesticides, an understanding of the chemical and physical forces acting upon these chemicals is important. One of the most effective means of detoxicating and degrading pesticides that find their way into soils is microbial action. Soil microorganisms are often the sole agencies involved in the removal of these potentially hazardous chemicals.

Pesticides are generally classed in groups according to their ability to control target organisms, the major categories being insecticides, herbicides, fungicides, and nematocides. At the present time much more is known about the metabolism of herbicides in soil than of the other pest-control chemicals. Although insecticides are generally more toxic to mammals and therefore pose a greater threat to public health, little is known about the pathways and the enzymes responsible for their initial decomposition in soil. The objective of this chapter is to report on the organisms known to be effective in degrading various herbicides, to present the proposed pathways of their metabolism, and to examine the isolated enzymes that are capable of altering the parent molecule so as to make the product innocuous.

13-2. PHENOXYALKANOIC ACIDS

The phenoxyalkanoic acids comprise a large number of herbicidal compounds that find extensive use in the control of broadleaf weeds. The structure of the phenoxyalkanoates consists, basically, of an aliphatic acid bonded to a benzene ring by an ether linkage. The best-known member of this class of compounds is 2,4-D; see Table 13-1 and Fig. 13-1. Other herbicides of the phenoxyalkanoate series are analogues of the 2,4-D, such various groups as chlorine, methyl, etc., being substituted at different positions on the ring or aliphatic acid moieties of various chain lengths. The structure and common names of several phenoxy herbicides is presented in Table 13-1.

Many, but not all, of the phenoxyalkanoates are metabolized by micro-organisms. The readily degraded compounds are the most readily lost from

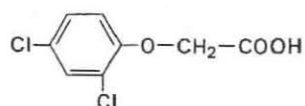


Fig. 13-1. Chemical structure of the phenoxyalkanoic acid herbicide 2,4-D(2,4-dichlorophenoxyacetic acid).

TABLE 13-1

Nomenclature of the Phenoxyalkanoate Herbicides

Common name	Chemical name
2,4-D	2,4-Dichlorophenoxyacetic acid
MCPA	2-Methyl-4-chlorophenoxyacetic acid
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid
2,4-DB	4-(2,4-Dichlorophenoxy)butyric acid
2,4-DP	2-(2,4-Dichlorophenoxy)propionic acid

soil. Of the more widely used herbicides 2,4-D is the most quickly degraded, MCPA persists somewhat longer, and soils receiving 2,4,5-T retain their toxicity longest. The rapid detoxication of the first two chemicals results from microbial action, as shown by the prolonged persistence when the herbicide-amended soil is treated with sodium azide (1). The potential for microbial degradation of pesticides in soil is governed by their chemical structure. Slight structural modifications often change a susceptible molecule into a resistant one, and vice versa. The most obvious characteristics that relate biodegradability to the molecular structure of the phenoxyalkanoates are the position of the ring halogens and the position of the linkage of the fatty acid to the benzene ring. The rate of destruction of the compounds studied so far is notably slow when the ring halogen is meta to the phenolic hydroxyl, or when the aliphatic moiety is linked to the ring at the α carbon. Phenoxy herbicides having side chains linked to the ring at the ω carbon and having no meta halogen are generally detoxicated rapidly (2). The possible reasons for such molecular recalcitrance have been discussed elsewhere (3).

Five possible metabolic steps or general processes concerned in the metabolism of the phenoxyalkanoates have been established (Fig. 13-2). These reactions are conversion of nonfatty-acid side chains to the corresponding fatty acids, β oxidation of compounds with long fatty-acid moieties,

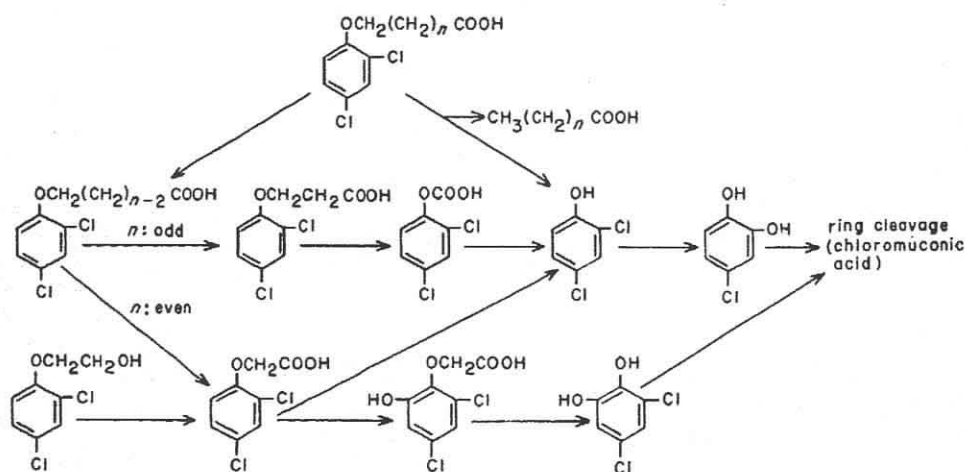


Fig. 13-2. Proposed pathways of phenoxyalkanoic acid metabolism by soil microorganisms.

yielding products with shorter alkanolic acid moieties, cleavage of the ether linkage between the side chain and the ring, ring hydroxylation, and ring cleavage. Several of these reactions have been shown to occur in soil.

Sesone (sodium 2,4-dichlorophenoxyethyl sulfate) is a compound that itself is not herbicidal; however, it can be converted to the active form microbiologically. The reaction in soil appears to result from an enzymatic mechanism involving the formation of 2,4-dichlorophenoxyethanol and 2,4-D (4,5).

The β oxidation sequence is a well-known biochemical pathway for the degradation of aliphatic hydrocarbons and fatty acids. Webley et al. (6) were the first to demonstrate its applicability to soil microorganisms. They observed the formation of the β -hydroxy derivative during the decomposition of 4-phenoxybutyrate, 4-(3-chlorophenoxy)butyrate, and 4-(2-chlorophenoxy)butyrate caused by *Nocardia opaca*. This actinomycete also produced phenoxyacetate from 4-phenoxybutyrate and 2-chlorophenoxybutyrate from 2-chlorophenoxypropionate, which suggests a typical β oxidation sequence. With paper chromatographic procedures and strains of *Nocardia*, *Pseudomonas*, and *Micrococcus* Taylor and Wain (7) verified this mechanism of side-chain degradation by showing the conversion of 5-phenoxyvalerate and 7-phenoxyheptanoate to 3-phenoxypropionate and the analogous conversion of 4-phenoxybutyrate and 6-phenoxyhexanoate to phenoxyacetate. The demonstration that β oxidation occurs not only in monocultures but also in natural soil was made by Gutenmann et al. (8), using 2,4-DB and the corresponding ω -substituted valerate, caproate, heptanoate, octanoate, nonanoate, decanoate, and undecanoate. Further support for the significance of this mechanism in soil can be found in the observation, made by Gutenmann and Lisk (9), that 2,4-dichlorophenoxyacetic acid is generated in 2,4-DB-treated soil and that the crotonate is then further degraded to 2,4-D.

A third mechanism of the initial attack on phenoxy herbicides involves the cleavage of the ether bond linking the benzene ring to the fatty acid. Whereas β oxidation results in no loss of phytotoxicity and may even lead to an increase in it, the cleavage of the ether linkage leads either to a loss of herbicidal activity or, in the case of compounds that require enzymatic activation, to a degradation sequence that prevents the chemical from attaining its potential for toxicity. The mechanism, demonstrated in a strain of *Flavobacterium*, converts an ω -substituted 2,4-dichlorophenoxyalkanoic acid into 2,4-dichlorophenol and the free fatty acid. By this mechanism the bacterium metabolizes all homologues in the series 3-(2,4-dichlorophenoxy)propionic acid to 8-(2,4-dichlorophenoxy)octanoic acid (10,11).

In the β oxidation of phenoxy herbicides with long aliphatic side-chain moieties the anticipated metabolic products of compounds with an even and with an odd number of carbon atoms in the side chain are, respectively, the phenoxyacetic and, possibly, the phenoxypropionic acids. It has been suggested that those substances with odd numbers of carbons in their aliphatic moieties are converted to phenoxypropionate, which in turn may undergo β oxidation yielding phenylcarbonate (phenoxyformate). The latter presumably is unstable and spontaneously gives rise to the corresponding phenol (6). In line with this suggestion are the findings of the production of phenolic compounds in cultures of *Nocardia coeliaca* provided with 5-phenoxyvalerate or 7-phenoxyheptanoate (7) and the finding of 2,4-dichlorophenol in soil treated with 2,4-dichlorophenoxyvalerate, heptanoate, nonanoate, and undecanoate (8).

The ether linkage of the phenoxyacetic acids is cleaved microbiologically. Soil pseudomonads have been reported to generate 2,4-dichlorophenol from 2,4-D, 2-methyl-4-chlorophenol from MCPA, and 4-chlorocatechol from 4-chlorophenoxyacetic acid (12). The significance of the phenol pathway in these bacteria is questionable. However, a soil *Arthrobacter* can use 2,4-D as its carbon source for growth, and phenol formation is its major means of degrading 2,4-D. This bacterium has generated 2,4-dichlorophenol from 2,4-D, but only trace amounts could be detected in the medium. Although the adaptation of the organism to 2,4-D led to an immediate induction of enzymes capable of catalyzing reactions with other phenoxyacetate substrates, the enzymes that initiated the destruction of the dichlorophenol were more specific and could not act upon all the products formed from the initial reactions. Thus, the 2,4-D-induced *Arthrobacter* metabolized phenoxyacetate and *p*-hydroxyphenoxyacetate but not the phenol and hydroquinone released. The latter compounds therefore accumulated in almost stoichiometric quantities (13). These findings indicate that cleavage of the ether linkage of 2,4-D is this bacterium's major mode of destroying the herbicide.

Ring hydroxylation also is common in microorganisms acting upon such compounds. This was first reported in detail by Byrde and Woodcock (14), their results showing that ω -substituted phenoxyalkanoates with no halogen substituents were hydroxylated in the *ortho* and *para* positions by *Aspergillus niger*. In replacement cultures of this fungus the major product of 2, 4-D metabolism was 5-hydroxy 2,4-D, and the major product of MCPA metabolism was 5-hydroxy MCPA (15). By contrast, the pseudomonads that grew upon these compounds produced 6-hydroxy 2,4-D and 6-hydroxy MCPA from 2,4-D and MCPA, respectively (12).

The steps involved in ring cleavage and dehalogenation have received little attention. It has been noted, however, that 4-chlorocatechol is

associated with the 2,4-dichlorophenol released from these herbicides by a *Flavobacterium*, the catechol presumably being due to the hydroxyl's replacing the chlorine group (100). Evans and his co-workers (12,16) showed that the catechols produced from 2,4-D, MCPA, and 4-chlorophenoxyacetate are cleaved to yield chloromuconic acids. These presumably are attacked by reactions involving a dehalogenation and the formation of simple organic acids that serve as substrates for a diverse group of soil microorganisms.

13-3. PHENYLCARBAMATES

The *N*-phenylcarbamates, phenylureas, and acylanilides are members of a large class of compounds known as phenylamides, many of which find wide application as herbicides for agronomic crops. All these compounds have the general formula $C_6H_5-NH-CO-R$. In the *N*-phenylcarbamates *R* is an alkoxy group, in the acylanilides it is an alkyl group, and in the phenylureas it is an alkylamino group. Although the *N*-phenylcarbamates and the phenylureas are similar in chemical structure, their routes of metabolism are quite different.

One of the important herbicidal *N*-phenylcarbamates is the isopropyl ester of the *N*-phenylcarbamate IPC; see Table 13-2 and Fig. 13-3.

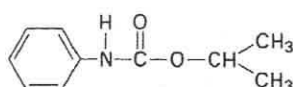


Fig. 13-3. Chemical structure of the phenylcarbamate herbicide IPC (isopropyl *N*-phenylcarbamate).

Some phytotoxic *N*-phenylcarbamates contain chlorine on the ring, whereas others are esterified with different alcohols. The structures of some typical phenylcarbamate herbicides and certain related chemicals are presented in Table 13-2.

TABLE 13-2
Nomenclature of the Phenylcarbamate Herbicides

Common name	Chemical name
IPC	Isopropyl <i>N</i> -phenylcarbamate
CIPC	Isopropyl <i>N</i> -(3-chlorophenyl)carbamate
CPPC	1-Chloro-2-propyl <i>N</i> -(3-chlorophenyl)carbamate
BCPC	<i>sec</i> -Butyl <i>N</i> -(3-chlorophenyl)carbamate
CEPC	2-Chloroethyl <i>N</i> -(3-chlorophenyl)carbamate
Barban	4-Chloro-2-butyryl 3-chlorocarbanilate

Results of field experiments indicate that the phenylcarbamates do not persist in soils. Soil microorganisms probably are responsible for decomposing most of these herbicides (17-25). Since barban, IPC, and CIPC disappear more rapidly from nonautoclaved than from autoclaved soils, the principal agents responsible for decomposition appear to be microorganisms (21,22,25). Little information exists on the relative persistences of these compounds in soil; CIPC is reported to be more persistent than IPC under similar conditions (26).

Recently microorganisms that degrade CIPC and CEPC have been isolated and described (27). The bacteria effective in degrading CIPC were identified as strains of *Pseudomonas striata*, *Flavobacterium*, *Agrobacterium*, and *Achromobacter*. Bacteria decomposing CEPC were identified as strains of *Achromobacter* and *Arthrobacter*. All the isolates utilized CIPC or CEPC as a sole source of carbon, as demonstrated by growth, 3-chloroaniline production, and the release of chloride ions. The CIPC-utilizing organisms also decomposed CEPC, but at a much slower rate; similarly, microorganisms active on CEPC metabolized CIPC more rapidly than CEPC. All the isolates were able to grow with IPC as a carbon source.

Both pure cultures and perfused soils metabolize ^{14}C -isopropyl- and ring-labeled CIPC (28-30). Radioactive CO_2 was evolved from both forms of the molecule in perfused muck soil. If the soils were initially herbicide-free, a lag period of 6 to 8 days, during which no $^{14}\text{CO}_2$ was evolved, followed the introduction of the labeled CIPC. Subsequently $^{14}\text{CO}_2$ was evolved rapidly and was accompanied by a release of chloride ion. Further additions of labeled CIPC were immediately and rapidly degraded, as evidenced by evolution of $^{14}\text{CO}_2$. A similar rapid rate of oxidation of second additions has been observed in soils receiving phenoxalkanoic acids and the chlorinated aliphatic acids (31,32). The decomposition of CIPC apparently proceeds by oxidation of the alcohol moiety and fragmentation of the phenyl ring. After the initial period of rapid metabolism there follows a slow steady evolution of $^{14}\text{CO}_2$ that may reflect the sorption or desorption of the pesticide from the soil particles.

The products of such metabolism due to intact microbial cells have not been identified, but with cell-free preparations of microorganisms the products 3-chloroaniline, CO_2 , and isopropyl alcohol are among the first detected. The results of feeding experiments with ^{14}C -labeled chloroaniline have been ambiguous, primarily because 3-chloroaniline is unstable under certain conditions: for example, its exposure to the atmosphere or to ultraviolet light results in the formation of a product that is chemically different. Preliminary results also indicate that dilute solutions of 3-chloroaniline sometimes inhibit the active organisms. Apparently, the compound is destroyed as rapidly as it is formed, and toxicity is not

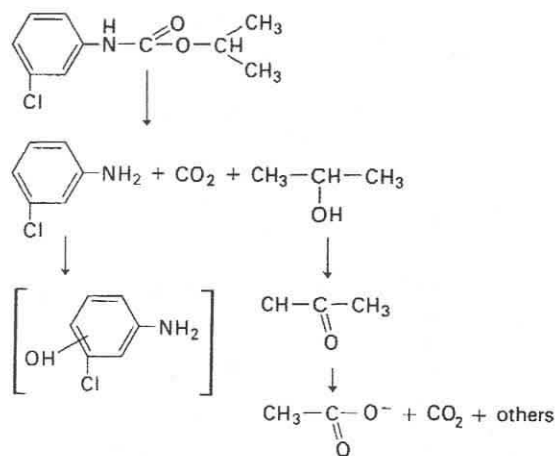


Fig. 13-4. Proposed pathway of CIPC metabolism by soil microorganisms.

apparent when the organism is provided with CIPC. On the basis of observations of other organisms that are capable of metabolizing isopropyl alcohol and 3-chloroaniline the scheme of CIPC dissimulation shown in Fig. 13-4 is proposed.

The enzymatic hydrolysis of CIPC and of related phenylcarbamates, by cell-free preparations of *Pseudomonas striata* isolated from soil, has been demonstrated by measuring the 3-chloroaniline production in saturated solutions of CIPC (33). Enzyme purification was achieved by a combination of precipitation with streptomycin sulfate and ammonium sulfate and by chromatography on DEAE cellulose. The purified enzyme catalyzed the decomposition of a variety of *N*-phenylcarbamate substrates, and it formed aniline, 3-chloroaniline, and 3,4-dichloroaniline from several phenylcarbamates structurally related to CIPC. The enzyme, however, was inactive on the insecticide 1-naphthyl *N*-methylcarbamate (carbaryl) and on the herbicide 3-(*p*-chlorophenyl)-1,1-dimethylurea (monuron), but it hydrolyzed two acylanilides, yielding the corresponding anilines.

Whether enzymatic cleavage proceeds by hydrolysis of the ester linkage or of the amide bond is unknown. Regardless of which bond is broken first, the products of the hydrolysis of CIPC would be essentially the same: that is, 3-chloroaniline, CO₂, and isopropyl alcohol. Other properties of this enzyme, such as pH optimum, substrate specificity, metal-ion requirement, inhibitors, and product verification, have been studied (33).

Electron density and steric properties affect the enzymatic hydrolysis of the *N*-phenylcarbamates. The relative acidity of several phenylcarbamates (Table 13-3) was correlated with the rate of hydrolysis by the partially

TABLE 13-3
Phenylcarbamates Examined for Effect of Acidity
on Rate of Enzymic Hydrolysis

Isopropyl <i>N</i> -phenylcarbamate
Isopropyl <i>N</i> -(2-chlorophenyl)carbamate
Isopropyl <i>N</i> -(3-chlorophenyl)carbamate
Propyl <i>N</i> -(3-chlorophenyl)carbamate
Ethyl <i>N</i> -(3-chlorophenyl)carbamate
Propynyl <i>N</i> -(3-chlorophenyl)carbamate
Isopropyl <i>N</i> -phenylthionocarbamate
1-Chloroisopropyl <i>N</i> -(3-chlorophenyl)carbamate
1,3-Dichloroisopropyl <i>N</i> -(3-chlorophenyl)carbamate

purified enzyme (33). As phenylcarbamates became more acid, the rate of hydrolysis increased. Those factors which increase the relative acidity of the phenylcarbamates appear also to influence the electron density in the vicinity of the carbonyl carbon undergoing nucleophilic attack. Any inductive effect within the molecule that tends to lower the density near this carbon should enhance the carbon's susceptibility to attack and therefore facilitate hydrolysis of the compound. A good correlation was established between relative acidity and rate of hydrolysis for seven of nine compounds investigated. Exceptions were the 1-chloroisopropyl and the 1,3-dichloroisopropyl *N*-3-chlorophenylcarbamates, in which the rates of enzymatic hydrolysis decreased as the relative acidity increased. The chlorines on the alcohol portion of these two phenylcarbamates may have sterically hindered the enzymatic attack in the vicinity of the carbonyl carbon. The implications of electronic effects on the approach of the substrate to the enzyme site (as measured by relative acidities), of resonance effects, of tautomerism, and, of hydrogen bonding between substrate and enzyme have not been studied in detail.

Structural effects on the rate of hydrolysis of phenylcarbamate herbicides differ between the intact microorganisms and the isolated enzyme. The purified enzyme from *Pseudomonas striata* hydrolyzed CIPC more rapidly than it did IPC, but the intact organism, though adapted for CIPC, metabolized IPC faster. The effect of chemical structure on adsorption and absorption of pesticides and their subsequent metabolism has received little attention so far.

13-4. PHENYLUREAS

Many dimethyl-substituted phenylurea derivatives possess herbicidal activity. The simplest member of this class of herbicides is 1,1-dimethyl-3-phenylurea, or fenuron (Fig. 13-5). Other herbicides in this group are

TABLE 13-4
Nomenclature of the Phenylurea Herbicides

Common name	Chemical name
Buturon	3-(<i>p</i> -Chlorophenyl)-1-methyl-1-(1-methyl-2-propynyl)urea
Chloroxuron	N'-(4-Chlorophenoxy)phenyl- <i>N,N</i> -dimethylurea
Diuron	3-(3,4-Dichlorophenyl)-1,1-dimethylurea
Fenuron	1,1-Dimethyl-3-phenylurea
Linuron	3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea
Monuron	3-(<i>p</i> -Chlorophenyl)-1,1-dimethylurea
Neburon	1-Butyl-3-(3,4-dichlorophenyl)-1-methylurea

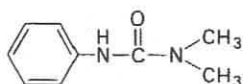


Fig. 13-5. Chemical structure of the phenylurea herbicide fenuron (1,1-dimethyl-3-phenylurea).

chlorinated at various positions on the ring or are substituted with other alkyl or alkoxy groups on the urea moiety. Some typical urea herbicides and their chemical formulae are presented in Table 13-4.

High organic-matter levels, warm temperatures, and proper moisture for microbial activity favor the rapid disappearance of phenylureas from soil (34). From comparisons of the rates of disappearance of these compounds from sterile and nonsterile soils it appears that the soil microflora are responsible, at least in part, for their degradation (34-43).

Studies of monuron methyl-labeled with ^{14}C and applied to a clay loam demonstrated that approximately 10% of the compound appeared as $^{14}\text{CO}_2$ in 90 days (36). Manometric evidence indicates that several soil microorganisms were capable of utilizing monuron as a sole source of carbon. The organisms included species of *Xanthomonas*, *Sarcina*, *Bacillus*, *Penicillium*, and *Aspergillus* (44). A species of *Pseudomonas* isolated from soil also oxidized monuron, but only in the presence of exogenous growth factors. On the basis of these studies it was concluded that the soil microflora contain phenylurea-decomposing organisms.

The rate of disappearance of phenylurea herbicides from soil is undoubtedly a function of the adsorption-desorption equilibrium. Phenylureas are absorbed strongly on soil particles (44-46). Geissbuhler et al. (41) calculated the percent adsorption of chloroxuron in soils in which rates of decomposition were being measured. The decomposition was more rapid in a sandy loam soil, in which 8% of the herbicide was in solution, than in

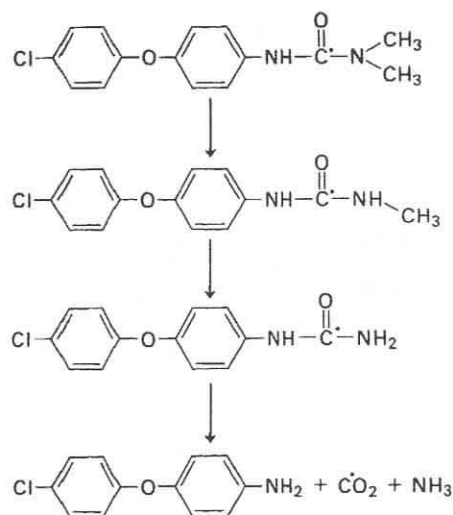


Fig. 13-6. Proposed pathway of chloroxuron metabolism by soil microorganisms. (Dots indicate position of ^{14}C in the parent compound and metabolites.) [From Geissbuhler et al. (41).]

a humus soil, in which 1 % of the herbicide was in solution. One explanation of the rapid disappearance of chloroxuron from this soil, which is low in organic matter, is that the adsorption-desorption equilibrium favors microbiological degradation.

Demethylation appears to be one of the initial steps in the metabolism of phenylureas in soil. The monoalkyl and the dealkylated analogues of monuron, diuron, and chloroxuron have been isolated and identified in a number of phenylurea-treated soils (41,43,47). The proposed pathway of chloroxuron decomposition in soil is shown in Fig. 13-6.

Studies of the fate of monuron and diuron indicate that the hydrolysis proceeds by successive removal of the two methyl groups, followed by hydrolysis of the remaining phenylurea, producing aniline (43). These metabolites have been identified in soil, but the proposed pathways have not been verified with any individual microbial isolates. Cotton roots grown in solution culture containing labeled diuron generate the same metabolites (47).

The process of oxidative removal of the *N*-alkyl groups from pesticide molecules is common to a number of biological systems. The reactions are generally not transmethyations, because formaldehyde usually is formed from the alkyl group. For example the insecticide schradan (octamethylpyrosphoramide) is oxidized to the corresponding hydroxymethyl derivative

in insects (48), and to heptamethyl pyrophosphoramidate and formaldehyde in rat-liver homogenates. Model systems of enzymatic dealkylation have been described, but the reaction has not been demonstrated with cell-free fractions of soil microorganisms.

Rat-liver microsomes capable of catalyzing the *N*-dealkylation of a number of *N,N*-dimethylphenylcarbamates were generally inactive on the *N,N*-dimethylphenylurea herbicides (49). In the presence of oxygen and reduced triphosphopyridine nucleotide all *N,N*-dimethylcarbamates yielded appreciable amounts of formaldehyde. The *N*-methylcarbamates are not readily oxidized in the same system, whereas the microsomal enzymes exhibited a low order of activity on the herbicides fenuron, monuron, and diuron. In general, the modification of the carbamate structure =N-CO-O- through a replacement of the ester oxygen with a nitrogen (i.e., formation of a urea analogue) or the substitution of a sulfur for either oxygen provides compounds that are poor sources for formaldehyde production in microsomal systems.

13-5. CHLORINATED ALIPHATIC ACIDS

A number of the simple chlorinated aliphatic acids are phytotoxic, but only two, dalapon (2,2-dichloropropionate) and TCA (trichloroacetate), are widely used for weed control. These compounds are commonly applied as the sodium salts.

Numerous studies of the persistence of these compounds in soil have been conducted, the results of which are summarized in several reviews (32,50,51). As a rule, dalapon at the usual application rates disappears from soil in 2 to 4 weeks, whereas TCA usually disappears within 4 to 12 weeks. Both compounds persist in the field for considerably longer periods at higher rates of application and have been used as soil sterilants for certain purposes.

The metabolism of the chlorinated aliphatic acids effected by soil microorganisms has been studied extensively (32,52-59). The compounds are generally very soluble, and simple procedures are available for determining their decomposition. Microbial activity is commonly followed by determining the release of halide ions. Kearney et al. (60) enumerate the bacteria, fungi, and actinomycetes capable of decomposing ten different chlorinated acetic, propionic, and butyric acids. The bacteria reported to decompose dalapon include species of *Bacillus*, *Pseudomonas*, *Agrobacterium*, *Arthrobacter*, *Micrococcus*, *Alcaligenes*, and *Flavobacterium*. Four fungi and two actinomycetes were observed to utilize it as a source of carbon. Microorganisms dissimulating TCA include strains of *Pseudomonas*, *Arthrobacter*, and *Trichoderma*.

The number, position, and type of halide substitution on the acetic,

propionic, and butyric acids affect their rate of decomposition. Generally, the more highly substituted compounds are degraded at a much slower rate. For example, Jensen (55) reported that the rates of decomposition of the chlorinated acetic acids in soil followed the order monochloroacetate > dichloroacetate > trichloroacetate. Likewise, Kaufman (58) found that increasing the number of chlorines on acetate, propionate, and butyrate compounds generally decreased the dehalogenating activity of nine soil isolates studied. The α -substituted propionic acids are generally attacked more rapidly than the β -substituted analogues. Isolates of *Pseudomonas* and *Nocardia* utilized 2-chloro-, 2-bromo-, 2-iodo-, and 2,2-dichloropropionate more readily than 2,3-dibromo- or 2,2,3-trichloropropionate (57). An enzyme catalyzing the dehalogenation of 2,2-dichloropropionate was inactive on any β -chlorosubstituted propionate (61,62). Differences also exist among organisms in their abilities to utilize fluoro-, chloro-, bromo-, and iodo-substituted acids (57). The number of atoms in the molecule also affects the rate: Kaufman (63,64) observed that halogenated propionic acids were decomposed more rapidly than the corresponding acetic and butyric acids, although one isolate was more effective on chlorinated butyrates than on either the corresponding propionates or acetates.

Although certain generalizations can be made about the influence of the chemical structure of the halogenated aliphatic acids on their susceptibility to microbial degradation, exceptions can be cited. For example, eight of nine microbial isolates grown on 2,2-dichloropropionate were more effective on the 3-chloropropionate than on the 2-chloropropionate (58). Moreover, Jensen (54) reported that, although a *Pseudomonas* species readily decomposed monochloroacetate, it was less active on 2-chloropropionate, had little effect on dichloroacetate or dichloropropionate, and was completely inactive on TCA. On the other hand, an unidentified non-spore-forming bacterium decomposed TCA quickly, attacked dichloroacetate only slightly, and was inactive on monochloroacetate and dalapon.

These dechlorination reactions may involve the replacement of the halogen with a hydroxyl group. The chlorinated acetic and propionic acids would thus yield the corresponding hydroxy and keto compounds, as proposed by Jensen (54). The limited information available on enzymatic mechanisms agrees with this hypothesis. Goldman (65) characterized an enzyme from a *Pseudomonas* species isolated from soil enrichments containing fluoroacetate as a source of carbon. The enzyme converted fluoroacetate to glycolate (Fig. 13-7). The purified enzyme also catalyzed the dehalogenation of chloroacetate and iodoacetate, but at a much slower rate. Further evidence that the hydroxylation mechanism is the initial step in the decomposition of halogenated aliphatic acids has been obtained with 3-bromopropionic acid: resting cells of a *pseudomonad* convert it to 3-hydroxypropionate (66).

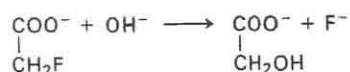


Fig. 13-7. Enzymatic conversion of fluoroacetate to glycolate by a *Pseudomonas* sp. isolated from soils. [From Goldman (65).]

The hydrolysis of dalapon was demonstrated with extracts of an *Arthrobacter* sp. isolated from soil (61,62). The enzyme catalyzed its conversion to pyruvic acid (Fig. 13-8). The partially purified enzyme was also capable of

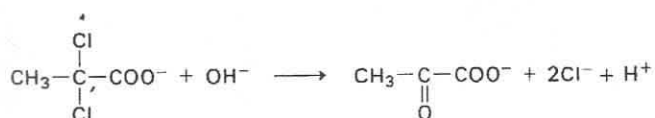


Fig. 13-8. Enzymatic conversion of 2,2-dichloropropionate to pyruvate by an *Arthrobacter* sp. isolated from soils. [From Kearney et al. (61).]

hydrolyzing dichloroacetic acid but was inactive on TCA and most β -chloro-substituted acids. The metabolism of TCA labeled with ^{14}C and added to cultures of an effective soil microorganism has been studied (28,29); when the compounds were labeled on the C-1 and C-2 atoms, the organism rapidly liberated $^{14}\text{CO}_2$ from both atoms, but the rate of liberation was more rapid from the C-1 than from the C-2. One of the products formed in the culture solution was identified as serine. The formation of Cl^- or $^{14}\text{CO}_2$ in active cultures was enhanced greatly by the addition of exogenous carbon sources.

Studies with an enzyme from an organism capable of liberating $^{14}\text{CO}_2$ from the C-1 but little from the C-2 of TCA also have been made (99). Partial purification of the responsible enzyme has been achieved by chromatography on Sephadex columns. Reduced triphosphopyridine nucleotide was essential for the liberation, and coenzyme A was stimulatory. The products of this metabolism have not yet been characterized.

Dalapon may be oxidized microbiologically without the liberation of chloride (57). The identity of the compound retaining the organic chlorine is unknown, however.

13-6. s-TRIAZINES

The phytotoxic activity of the 2-chloro-s-triazines was first demonstrated by Gysin and Knusli (67). The herbicidal s-triazine compounds usually contain alkylamino substituents at the 4 and 6 positions of the triazine ring and a chlorine, an alkoxy, or a mercapto substituent at the 2 position. Simazine (Fig. 13-9) and atrazine have found particularly wide application

TABLE 13-5
Nomenclature of the *s*-Triazine Herbicides

Common name	Chemical name
Simazine	2-Chloro-4,6-bis(ethylamino)- <i>s</i> -triazine
Atrazine	2-Chloro-4-ethylamino-6-isopropylamino- <i>s</i> -triazine
Atraton	2-Methoxy-4-ethylamino-6-isopropylamino- <i>s</i> -triazine
Propazine	2-Chloro-4,6-bis(isopropylamino)- <i>s</i> -triazine
Prometone	2-Methoxy-4,6-bis(isopropylamino)- <i>s</i> -triazine
Prometryne	2,4-Bis(isopropylamino)-6-methylmercapto- <i>s</i> -triazine
Ipazine	2-Chloro-4-diethylamino-6-isopropylamino- <i>s</i> -triazine

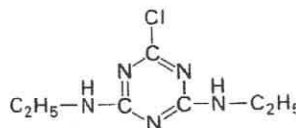


Fig. 13-9. Chemical structure of the *s*-triazine herbicide simazine (2-chloro-4,6-bis(ethylamino)-*s*-triazine).

as a selective herbicide for the control of many weeds in corn. Typical *s*-triazine herbicides and their chemical nomenclature are presented in Table 13-5.

The first investigations of the metabolism of the *s*-triazines were made with drugs applied to animals. Formoguanamine (2,4-diamino-*s*-triazine) was converted to ammelide (2,4-bis(hydroxy)-6-amino-*s*-triazine) in man (68). A similar reaction was noted in mice fed with 2,4,6-triethylenemelamine (69); in this case cyanuric acid (2,3,4-trihydroxy-*s*-triazine) was the principal urinary metabolite. These early studies with mammals suggested that the *s*-triazine ring remained intact, whereas the side chain was acted upon.

The conversion of simazine to hydroxysimazine (2-hydroxy-4,6-bis(ethylamino)-*s*-triazine) occurs rapidly in strong acids at room temperature. This reaction has been employed for the quantitative determination of 2-chloro-*s*-triazines extracted from plants and soil, because the hydroxy derivative absorbs ultraviolet light more strongly than the parent material. The conversion of simazine to hydroxysimazine also appears to be a major mechanism of detoxication in plants and soil. Hydroxysimazine was the only labeled metabolite isolated by extraction of four soils initially treated with ring-labeled simazine and maintained at field capacity at $30 \pm 2^\circ\text{C}$ for 32 weeks (70,102).

In the plant detoxication reaction the hydroxysimazine forms non-enzymatically. The reaction involves a benzoxazine derivative (71); see Fig. 13-10. The resistance of certain plants to simazine appears to be related

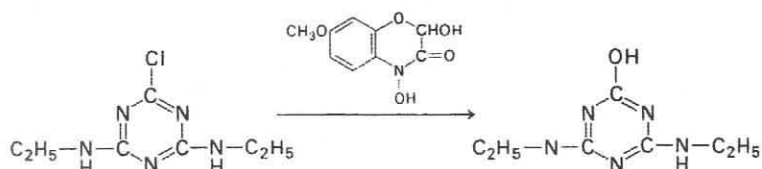


Fig. 13-10. Nonenzymatic conversion of simazine to hydroxysimazine catalyzed by a benzoxazine derivative isolated from plants. [From Hamilton and Moreland (71).]

to the concentration of the benzoxazine derivatives, the correlation being found in the plant tissues (72). This does not explain, however, all selective differences; other mechanisms are probable. At present it is unknown what catalyzes the conversion of simazine to hydroxysimazine in soils. Preliminary studies indicate that the conversion is probably nonbiological, inasmuch as it was unaffected by the microbial inhibitor sodium azide (70,101).

Soil receiving ring-labeled simazine, atrazine, and propazine does not release significant amounts of labeled CO_2 ; which suggests that ring rupture occurs very slowly. By contrast, ring-labeled ipazine has been attacked to a significant extent (63,64,73). As a rule, however, the *s*-triazine ring remains intact for extended periods of time in soil and is not very susceptible to microbial cleavage.

A number of soil fungi have been reported to metabolize the ethylamino group in simazine (74,75). Dealkylation was demonstrated in culture solutions of *Aspergillus fumigatus* containing either chain-labeled or ring-labeled simazine; the $^{14}\text{CO}_2$ was evolved only from the chain-labeled compound. This observation suggests that the ethyl or ethylamino group was removed but that the *s*-triazine ring was not cleaved. Subsequently, Couch et al. (76) demonstrated that four soil fungi metabolized ethyl-labeled simazine but not ring-labeled atrazine. A metabolite was detected in *A. fumigatus* cultures that did not possess the same relative mobilities as hydroxysimazine on paper chromatograms. The compound was identified by infrared spectra, nuclear magnetic resonance, and mass spectra as 2-chloro-4-amino-6-ethylamino-*s*-triazine (32). A second metabolite was detected in *A. fumigatus* cultures, that contained an intact ring and a chlorine at the 2 position. In addition, a small amount of ammelide was detected. The pathways proposed for the degradation of simazine in soil and in higher plants are shown in Fig. 13-11.

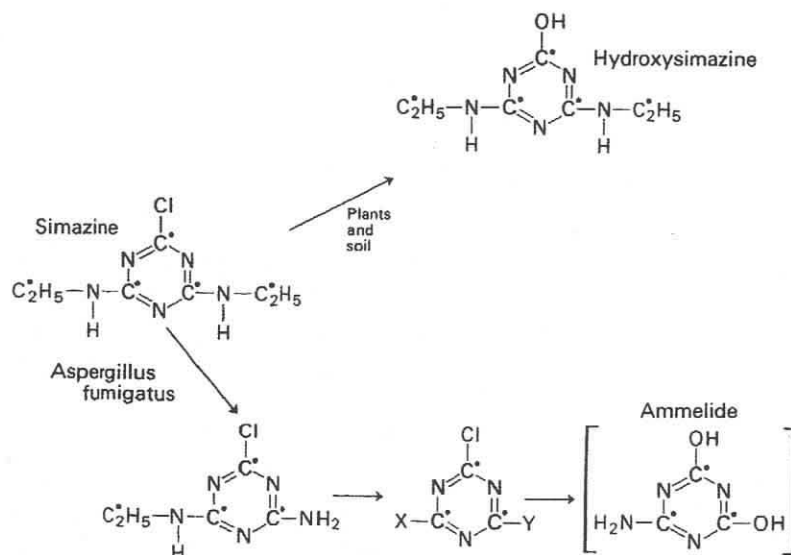


Fig. 13-11. Proposed pathway of simazine degradation in soils. (Dots indicate position of ^{14}C in the parent compound and metabolites). [From Kearney et al. (32).]

Side-chain degradation also has been observed in higher plants (77). Atrazine fed to pea plants yielded a new metabolite, which was identified as 2-chloro-4-amino-6-isopropylamino-*s*-triazine. Apparently the ethyl is removed more readily than the isopropyl group.

13-7. TOLUIDINES AND ACETAMIDES

One of the new herbicides that is finding wide application in cotton and soybeans is trifluralin (α, α, α -trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine). For best results it is recommended that trifluralin be incorporated in soils simultaneously with, or immediately after application to prevent loss of its activity. When incorporated, general soil persistence studies indicate that trifluralin decomposes slowly but will not carry over to succeeding crops when used as recommended. Metabolic studies with ^{14}C -labeled trifluralin in soils indicated that the first reaction associated with trifluralin decomposition appears to be the removal of the *N*-propyl group on the amine nitrogen (78). Decomposition proceeds further by the removal of the second *N*-propyl group from the aromatic amine. It is then postulated that the decomposition pathway is as shown in Fig. 13-12. As a logical

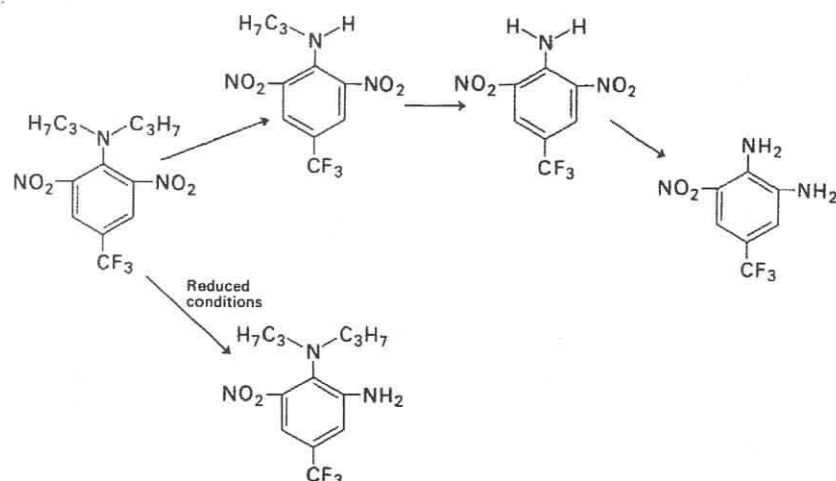


Fig. 13-12. Proposed pathway of trifluralin metabolism in soils. [From Parka and Tepe (78).]

extension of the reactions shown, one might expect that the remaining nitro group could be reduced and conceivably produce a compound such as the triaminotrifluoromethyl-substituted derivative.

Preliminary soil studies under normal soil moisture conditions indicate that soil microorganisms probably play a part in the inactivation of trifluralin in soil. However, under certain anaerobic field conditions only one metabolite has been isolated from the soils (78). Subsequent isolation and identification of this compound reveal it to be the intact trifluralin with one of the nitro groups reduced to the amine. Circumstances under which this metabolite is found in the field lead one to believe that anaerobic conditions are necessary for the reduction of the nitro group to take place.

Detailed studies of trifluralin decomposition by isolated soil microorganisms have not yet been made. The principal problem has been the solubility of the parent compound, which is less than 1 ppm. It is conceivable that insufficient energy is available to the microorganism from the trifluralin in solution, and that the rate of solubilization is too slow to permit rapid proliferation of microbial isolates.

Detailed metabolic studies with diphenamid (*N,N*-dimethyl-2,2-diphenylacetamide) have yet to be conducted in soils. Some recent studies with the metabolism of diphenamid in strawberry plants have been reported (79). The major product found in strawberries grown in soils treated with diphenamid labeled in the carbonyl position was the parent compound. The major metabolite in strawberries was the *N*-methyl-2,2-diphenylacetamide, and small quantities of the 2,2-diphenylacetic acid and hydroxylated derivatives of diphenamid were also detected.

13-8. AMITROLE

The fate of amitrole (3-amino-1,2,4-triazole) in soil is governed principally by the presence and activity of soil microorganisms, the adsorption on soil constituents, and nonbiological reactions (80). The rapid evolution of $^{14}\text{CO}_2$ from soils treated with ^{14}C -labeled amitrole indicates that microorganisms may participate in degradation, (73,81), but the isolation and identification of these microorganisms have not been reported. Ashton (82) investigated the fate of amitrole, labeled with ^{14}C in the 5 position, in sterile and nonsterile Yolo sandy loam and attributed the decomposition to microbiological activity. $^{14}\text{CO}_2$ was the major metabolic product, but at least thirteen additional labeled compounds were detected. One or more of them were tenaciously bound to the soil and appeared to resist further degradation.

Sund (83) proposed that the disappearance of amitrole from some soils was due largely to a combination of complexing and adsorbing actions. Ercegovich and Frear (84,102) observed a rapid rate of disappearance from both methylbromide-sterilized and nonsterile Hagerstown silt loam soil. The disappearance from a steam-sterilized soil was somewhat slower; however, it is known that steam sterilization alters the chemical and physical characteristics of soil. Such observations indicate that, at least in some soils, microorganisms are not primarily responsible for the disappearance of amitrole.

13-9. DIPYRIDYLS

Paraquat (1,1'-dimethyl-4,4'-bipyridinium salt) is a desiccant and herbicide recommended for aquatic weed control. Chemically, paraquat may be regarded as two molecules of pyridine methiodide bonded together through the para positions. It has been proposed that the paraquat cation must be converted to a free radical before herbicidal activity can be attained (85). Although the bipyridinium herbicides are active on aquatic weeds (86,87), they are so tenaciously adsorbed by base exchange on some soil particles that they are completely inactive in soils (88,89). Nonetheless, the metabolism of paraquat has recently been demonstrated (90) in culture solutions of an unidentified bacterium isolated from enriched soils. Studies of ^{14}C -labeled paraquat by means of thin-layer electrophoresis yielded two products that were tentatively identified by cochromatography. The two metabolites, 1-methyl-4,4'-bipyridinium ion and 1-methyl-4-carboxypyridinium ion, suggest that the pathway of degradation is first the demethylation of the parent molecule, then ring cleavage of one of the heterocyclic rings, and finally formation of the carboxylated *N*-methyl pyridinium ion (Fig. 13-13). The latter compound has been identified as one of several

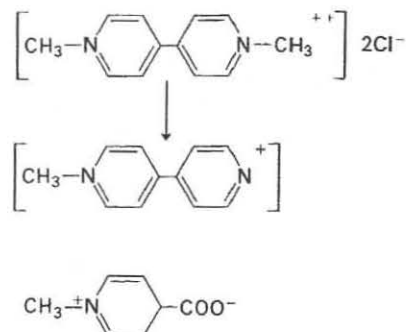


Fig. 13-13. Proposed pathway of paraquat metabolism by an unidentified bacterium isolated from soils. [From Bozarth et al. (90).]

degradation products of paraquat exposed to ultraviolet light (91). Similar detoxications may be carried out by aquatic microorganisms.

Thus far primary emphasis in this chapter has been given to the biological detoxication reactions. Nonbiological degradations also are responsible for removing many toxins from the soil, but their magnitude and their importance in relation to the enzyme-catalyzed reactions are not well understood. The photodecomposition of herbicides on soil surfaces immediately after application is probably one of the most studied of the reactions associated with the nonbiological degradation of pesticides. Volatilization, leaching, adsorption, absorption, and runoff may all contribute to the loss of herbicides from soil. Although these factors do not fall within the context of the subject matter presented here, it is essential that they be kept in mind in interpreting data on persistence and rate of degradation. Of greater importance in a discussion of this type are factors affecting soil microbial activity.

13-10. ECOLOGY AND MICROBIOLOGY OF HERBICIDE DEGRADATION

The rate at which a pesticide decomposes in soil is subject to a number of variables acting on and within the soil microorganisms and on the molecule undergoing metabolism. At least five conditions must be met before a herbicide molecule may be microbiologically degraded (92); these are as follows.

1. An organism that is effective in metabolizing a herbicide molecule must live in soil or be capable of developing therein.
2. The compound must be in a form suitable for microbial degradation.
3. The chemical must be available to the organism.
4. The compound usually must be capable of inducing formation of the enzyme or enzymes appropriate for detoxication, because most enzymes

require induction. Low solubility or low concentration of the pesticide or barriers to permeability within the organism may be associated with lack of induction.

5. Environmental conditions such as soil pH, temperature, and organic matter must be suitable if the microorganism is to proliferate and the enzyme to operate.

If any of these conditions are not met, the pesticide will not be readily decomposed and may persist in the soil for long periods.

The mechanism by which a soil microbial population develops the capacity to degrade a pesticide is not completely understood. Audus (31) has suggested two major possibilities: chance mutation and adaptivity of enzymes. These processes have been adequately discussed elsewhere (3,31,32,92). More recently it has become apparent that certain herbicides are degraded by "constitutive soil microorganisms," that is, soil organisms that require little or no adaptation prior to their active degradation of the chemicals (63,64). In any case, organisms having the ability to utilize an applied pesticide as a carbon or energy source should proliferate rapidly in the absence of substrate competition. Under such conditions one might expect to find in the soil population large numbers of the organism that could be readily isolated with the aid of selective media containing the pesticide as a sole or major source of energy. Such has not always been the case, however, as evidenced in attempts at isolating microorganisms effective in degrading phenylureas, aminotriazoles, and thiocarbamates.

Anaerobiosis has been associated with the long life of certain compounds in the natural environment (3,92). The great resistance to microbial degradation in anaerobic systems may be due to the biogenesis of microbial toxins or the need for molecular oxygen in enzymatic degradation. Little information exists on the effect of anaerobiosis on pesticide degradation. Recently, however, Agundis and Behrens (93) observed that atrazine disappeared from soil more rapidly under anaerobic conditions than under aerobic conditions, and Parka and Tepe (78) observed the same in the case of trifluralin.

Although much is known concerning the effect of adsorption-desorption equilibria on the microbial metabolism of certain organic molecules, little is known about the effect of adsorption on the microbial degradation of pesticides. Adsorption of a pesticide onto soil particles may adversely affect the availability of the compound to microorganisms, and so may the sorption of extracellular enzymes involved in the detoxication sequence. Adsorption-desorption phenomena show a more rapid degradation of chloroxuron in soils deficient in organic matter than in soils rich in it (41). The evolution of $^{14}\text{CO}_2$ from *Aspergillus fumigatus* growing in solutions of simazine chain-labeled with ^{14}C was slower in the presence of a 200-mesh

bentonite clay than in the absence of such; presumably, the clay limited the availability of the compound to the organism.

The chemical and physical characteristics of soil that are known to affect soil microorganisms are known also to affect the rates of disappearance of herbicides from soils. However, these factors must be considered independently as well as jointly, for their actions in the one respect may well be independent of those in the other. Thus, for example, an increased decomposition of a pesticide in soil may not necessarily result from increased microbial activity per se, but may reflect an increased availability of the chemical to the organism.

Microbial activity increases with increasing temperature (94). The degradation (95,96) and availability (96,97) of certain herbicides also increase with increasing temperature. In general, soil microorganisms are more active in soils with high organic matter levels than in soils with low. The influence of soil organic matter on the persistence of pesticides in soil however, is not completely understood. Certain pesticides are degraded rapidly in soils having high organic-matter levels (58); others are degraded slowly (41,98).

Soil pH, cation-exchange capacity (58,96,97), and moisture content (80,98) affect the availability and persistence of certain herbicides in soil, and they also affect the activities of soil microorganisms. In general, where microbial activity is high, degradation occurs more rapidly.

13-11. CONCLUSION

Soil microorganisms constitute a diverse and ubiquitous group of organisms. Actinomycetes, fungi, and bacteria are primarily responsible for the biological decomposition of herbicides in soils. Biochemical reactions known to occur in the microbial decomposition of various herbicides include β oxidation, cleavage of ether linkages, ring hydroxylation, ring cleavage, ester hydrolysis, dehalogenation, and *n*-dealkylation.

Enzymes responsible for catalyzing several of these reactions have been described with cell-free systems isolated from adapted soil microorganisms. There is some evidence that they are induced by the herbicides in soils and are not normal constituents of the organism. The rate-determining factors of the reactions are characteristic of the soil, the microorganism, and the herbicide molecule. It is difficult to determine what contribution each of these variables makes to the length of life of a herbicide in a system as heterogeneous as soil. *In vitro* studies with pure and mixed cultures of microorganisms and isolated enzymes have shed considerable light on the effect of molecular configuration on rates of decomposition. The number, position, and type of substituents strongly influence the susceptibility of a

herbicide molecule to microbial attack. These effects are apparent in nearly all classes of herbicides.

Far less well understood are the electronic and steric influences of the herbicides on decomposition rates. The effects of resonance, tautomerism, electron density, and hydrogen bonding on these enzyme-catalyzed reactions are virtually unexplored. Finally, often when any consideration of these empirical observations is applied to soil persistence, the results are disconcerting, because the physical environment in which a herbicide molecule exists in soil may not be ideally suited to microbiological decomposition.

REFERENCES

1. L. J. Audus, *Plant Soil*, **3**, 170 (1951).
2. K. Burger, I. C. MacRae, and M. Alexander, *Soil Sci. Soc. Am. Proc.*, **26**, 243 (1962).
3. M. Alexander, *Advan. Appl. Microbiol.*, **7**, 35 (1965).
4. L. J. Audus, *Nature*, **171**, 523 (1953).
5. A. J. Vlitos and L. J. King, *Nature*, **171**, 523 (1953).
6. D. M. Webley, R. B. Duff, and V. C. Farmer, *J. Gen. Microbiol.*, **18**, 733 (1958).
7. H. F. Taylor and R. L. Wain, *Proc. Roy. Soc. (London)*, **B156**, 172 (1962).
8. W. H. Gutenmann, M. A. Loos, M. Alexander, and D. J. Lisk, *Soil Sci. Soc. Am. Proc.*, **28**, 205 (1964).
9. W. H. Gutenmann and D. J. Lisk, *J. Agr. Food Chem.*, **12**, 46, 322 (1964).
10. I. C. MacRae and M. Alexander, *J. Bacteriol.*, **86**, 1231 (1963).
11. I. C. MacRae and M. Alexander, *Argon. J.*, **56**, 91 (1964).
12. W. C. Evans, J. K. Gaunt, and J. I. Davies, *Comm. Vth Intern. Congr.*
13. M. A. Loos, R. N. Roberts, and M. Alexander, *Bact. Proc.*, **65**, 3 (1965).
14. R. J. W. Byrde and D. Woodcock, *J. Biochem.*, **65**, 682 (1957).
15. J. K. Faulkner and D. Woodcock, *Nature*, **203**, 865 (1964).
16. J. K. Gaunt and W. C. Evans, *J. Biochem.*, **79**, 25 (1961).
17. H. R. DeRose, *Botan. Gaz.*, **107**, 583 (1946).
18. J. W. Mitchell and P. C. Marth, *Science*, **106**, 15 (1947).
19. A. R. Wolcott and R. F. Carlson, *Mich. State Univ. Agr. Exptl. Sta. Quart. Bul.*, **30**, 218 (1947).
20. R. J. Weaver, *Botan. Gaz.*, **109**, 276 (1948).
21. A. S. Newman, H. R. DeRose, and H. T. DeRigo, *Soil Sci.*, **66**, 393 (1948).
22. V. H. Freed, *Weeds*, **1**, 48 (1951).
23. L. F. Stevens and R. F. Carlson, *Proc. Northeastern Weed Control Conf.*, **6**, 33 (1952).
24. R. E. Ogle and G. F. Warren, *Weeds*, **3**, 257 (1954).
25. K. P. Dubrovin, *Crops Soils*, **14**, 26 (1962).
26. H. R. DeRose, *Agron. J.*, **43**, 139 (1951).
27. D. D. Kaufman and P. C. Kearney, *Appl. Microbiol.*, **13**, 443 (1965).
28. P. C. Kearney and D. D. Kaufman, *American Chemical Society, 149th Meeting*, Detroit, Mich., 1965.
29. P. C. Kearney and D. D. Kaufman, *American Chemical Society, 150th Meet.*, Atlantic City, N.J., 1965.
30. P. C. Kearney and D. D. Kaufman, *Science*, **147**, 740 (1965).
31. L. J. Audus, *Herbicides and the Soil* (review), Blackwell, Oxford, England, 1960.
32. P. C. Kearney, D. D. Kaufman, and T. J. Sheets, *J. Agr. Food Chem.*, **13**, 369 (1965).

33. P. C. Kearney, *J. Agr. Food Chem.*, **13**, 561 (1965).
34. T. J. Sheets, *J. Agr. Food Chem.*, **12**, 30 (1964).
35. G. D. Hill, *Proc. Southern Weed Conf.*, **7**, 76 (1954).
36. G. D. Hill, J. W. McGahen, H. M. Baker, D. W. Finnerty, and C. W. Bingeman, *Agron. J.*, **47**, 93 (1955).
37. T. J. Sheets, *Weeds*, **6**, 413 (1958).
38. T. J. Sheets, *Weeds*, **7**, 442 (1958).
39. R. P. Upchurch, *Weeds*, **6**, 161 (1958).
40. R. P. Upchurch and D. D. Mason, *Weeds*, **10**, 9 (1962).
41. H. Geissbuhler, C. Haselback, H. Aebi, and L. Ebner, *Weed Res.*, **3**, 277 (1963).
42. H. D. Dubey and J. F. Freeman, *Soil Sci.*, **97**, 334 (1964).
43. R. L. Dalton, A. W. Evans, and R. C. Rhodes, *Proc. Southern Weeds Conf.*, **18**, 72 (1965).
44. G. D. Hill, *Weed Soc. Am. Abstr.*, 1956, p. 42.
45. H. R. Sherburne and V. H. Freed, *J. Agr. Food Chem.*, **2**, 937 (1954).
46. Q. H. Yuen and H. W. Hilton, *J. Agr. Food Chem.*, **10**, 386 (1962).
47. J. W. Smith and T. J. Sheets, *Weed Soc. Am. Abstr.*, **6**, 39 (1966).
48. R. D. O'Brien, *Toxic Phosphorous Esters*, Academic Press, New York, 1960.
49. E. Hodgson and J. E. Casida, *Biochem. Pharmacol.*, **8**, 179 (1961).
50. B. J. Thiggs, *Down to Earth*, **18**, 7 (1962).
51. J. K. Keasure, *J. Agr. Food Chem.*, **12**, (1), 40 (1964).
52. H. L. Jensen, *Can. J. Microbiol.*, **3**, 205 (1956).
53. H. L. Jensen, *Can. J. Microbiol.*, **3**, 151 (1957).
54. H. L. Jensen, *Nature*, **180**, 1416 (1957).
55. H. L. Jensen, *Acta Agr. Scand.*, **10**, 83 (1960).
56. L. A. Magee and A. R. Colmer, *Can. J. Microbiol.*, **5**, 255 (1959).
57. P. Hirsch and M. Alexander, *Can. J. Microbiol.*, **6**, 241 (1960).
58. D. D. Kaufman, *Can. J. Microbiol.*, **10**, 843 (1964).
59. M. L. Beall, P. C. Kearney, and D. D. Kaufman, *Weed Soc. Am. Abstr.*, 1964, p. 12.
60. P. C. Kearney, C. I. Harris, D. D. Kaufman, and T. J. Sheets, *Adv. Pest Control Res.*, **6**, 1 (1965).
61. P. C. Kearney, D. D. Kaufman, and M. L. Beall, Jr., *Biochem. Biophys. Res. Commun.*, **14**, 29 (1964).
62. P. C. Kearney, D. D. Kaufman, and M. L. Beall, Jr., *Weed Soc. Am. Abstr.*, 1964, p. 11.
63. D. D. Kaufman, *Agron. J.*, **57**, 85 (1965).
64. D. D. Kaufman, unpublished data, 1965.
65. P. Goldman, *J. Biol. Chem.*, **240**, 3434 (1965).
66. C. E. Castro and E. W. Bartnicki, *Bact. Proc.*, **65**, 10 (1965).
67. H. Gysin and E. Knusli, *Advan. Pest Control Res.*, **3**, 289 (1960).
68. W. L. Lipschitz and E. Stokey, *J. Pharmacol., Exptl. Therap.*, **92**, 131 (1948).
69. M. V. Nadkarni, E. I. Goldenthal, and P. R. Smith, *Cancer Res.*, **14**, 559 (1954).
70. C. I. Harris, *Weed Res.*, **5**, 275 (1965).
71. R. H. Hamilton and D. E. Moreland, *Science*, **135**, 373 (1962).
72. R. H. Hamilton, *J. Agr. Food Chem.*, **12**, 14 (1964).
73. I. C. MacRae and M. Alexander, *J. Agr. Food Chem.*, **13**, 72 (1965).
74. D. D. Kaufman, P. C. Kearney, and T. J. Sheets, *Science*, **142**, 405 (1963).
75. D. D. Kaufman, P. C. Kearney, and T. J. Sheets, *J. Agr. Food Chem.*, **13**, 238 (1965).
76. R. W. Couch, J. V. Gramlich, D. E. Davis, and H. H. Funderburk, Jr., *Proc. Southern Weed Conf.*, **18**, 623 (1965).

77. R. H. Shimabukuro, R. E. Kadence, and D. S. Frear, *Weed Soc. Am. Abstr.*, **6**, 37 (1966).
78. S. J. Parka and J. E. Tepe, *Weed Soc. Am. Abstr.*, 1966, p. 40.
79. T. Golab, R. Herberg, and J. Tepe, *American Chemical Society*, 150th Meeting, Atlantic City, N.J. 1965.
80. C. D. Ercegovich and D. E. H. Frear, *J. Agr. Food Chem.*, **12**, 26 (1964).
81. D. D. Bondarenko, *Proc. Northeastern Weed Control Conf.*, **15**, 5 (1958).
82. F. M. Ashton, *Weeds*, **11**, 167 (1963).
83. K. A. Sund, *J. Agr. Food Chem.*, **4**, 57 (1956).
84. C. D. Ercegovich, Ph.D. thesis, Pennsylvania State Univ., University Park, Pa., 1957.
85. G. C. Mees, *Ann. Appl. Biol.*, **48**, 601 (1960).
86. R. D. Blackburn and L. W. Weldon, *Proc. Southern Weed Conf.*, **16**, 365 (1963).
87. L. W. Weldon and R. D. Blackburn, *Proc. Southern Weed Conf.*, **16**, 365 (1963).
88. A. Calderbank, *J. Agr. Veter. Chem.*, **1**, 197 (1960).
89. R. F. Homer, G. C. Mees, and T. E. Tompkinson, *J. Sci. Food Agr.*, **11**, 309 (1960).
90. G. A. Bozarth, H. H. Funderburk, Jr., and E. A. Curl, *Weed Soc. Am. Abstr.*, 1966, p. 55.
91. H. H. Funderbunk, Jr., N. S. Negi, and J. M. Lawrence, *Weed Soc. Am. Abst.*, 1966, p. 41.
92. M. Alexander, *Soil Sci. Soc. Am., Proc.* **29**, 1 (1965).
93. O. Agundis and R. Bhrens, *Weed Soc. Am. Abstr.*, 1966, p. 70.
94. M. Alexander, *Introduction to Soil Microbiology*, Wiley, New York, 1961, p. 472.
95. P. Bruschel, *Weed Res.*, **1**, 131 (1961).
96. R. E. Talbert and O. H. Fletchall, *Weeds*, **13**, 46 (1965).
97. C. I. Harris and G. F. Warren, *Weeds*, **12**, 120 (1964).
98. K. Holly and H. A. Roberts, *Weed Res.*, **3**, 1 (1963).
99. P. C. Kearney, D. D. Kaufman, D. von Endt, and F. S. Bernstein, *Weed Soc. Am. Abstr.*, 1966, p. 34.
100. I. C. MacRae, M. Alexander, and A. D. Rovira, *J. Gen. Microbiol.*, **32**, 69 (1963).
101. C. I. Harris and T. J. Sheets, *Weeds*, **13**, 215 (1965).
102. C. D. Ercegovich and D. E. H. Frear, *J. Agr. Food Chem.*, **12**, 26 (1957).